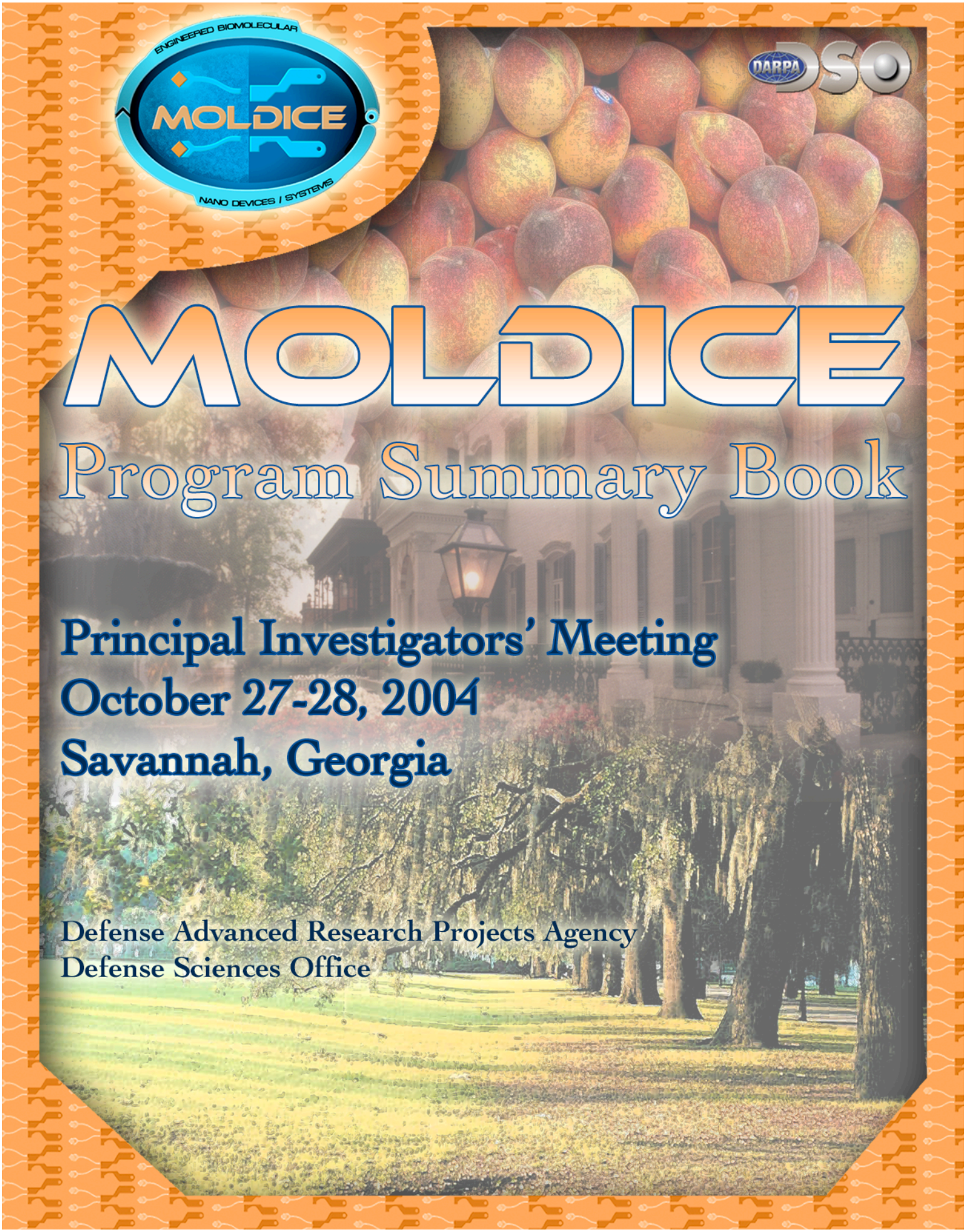
ENGINEERED BIOMOLECULAR
MOLDICE
NANO DEVICES / SYSTEMS
DARPA DSO
MOLDICE
Program Summary Book
Principal Investigators' Meeting
October 27-28, 2004
Savannah, Georgia
Defense Advanced Research Projects Agency
Defense Sciences Office

FOREWORD

This summary book reflects the current status of the DARPA MOLDICE (Engineered Bio-Molecular Nano Devices/Systems) Program, which began in FY2003. It is designed as a quick reference guide that describes the goals, the recent accomplishments and the milestones of the projects in the program. I would like to thank the Principal Investigators and their team members for their contributions to this book. I also wish to thank the staff of System Planning Corporation for their diligent efforts in compiling this book.

October 2004
Anantha Krishnan, Sc. D.
MOLDICE Program Manager
DARPA/DSO
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Program Summaries

Program Summary

Background: Ongoing research in nanotechnology is starting to demonstrate controlled fabrication of high quality nanostructures (nanoparticles, nanotubes, nanopores, etc.) that are capable of interacting with biology at the molecular scale. Significant recent accomplishments in biology and surface chemistry have also demonstrated programmed assembly of engineered molecular structures with excellent control on spatial distribution and orientation. Biological systems show remarkable sensitivity, specificity and efficiency due to the selective evolution of molecular mechanisms over several millions of years. It is anticipated that the engineering of hybrid molecular assemblies involving bio-molecules would enable the exploitation of these unique aspects of biological systems while affording the control that is possible through nanotechnology. This would lead to 'smart' bio-molecular assemblies with new functionalities (e.g., nano-sensors, nano-power generators, nano-chemical factories, etc.) and significant advantages (over conventional engineering systems) in terms of performance, size, power consumption, efficiency and ease of fabrication. The development of this technology will enable a new generation of integrated mechanical/optical/electronic/chemical devices at the nanoscale. This would lead to 'smart' large-scale integrated systems consisting of several such devices that demonstrate the attributes of adaptivity/reconfigurability, feedback control, fault tolerance and compensation at the system scale.

Program Objective: Biological systems have well defined sensing units, signal processing units and actuation sub-systems that determine responses to specific stimuli. While significant effort has gone into understanding the sensing systems of biology (e.g., receptor and transmembrane proteins), the intra-cellular signal processing system is still the subject of many ongoing research efforts. The objective of this research is to develop hybrid bio-molecular devices/systems that use biological units (e.g., Protein Ion Channels/Nanopores, G-Protein Coupled Receptors, etc.) for performing the sensing function but use silicon circuitry to accomplish the signal processing. Innovative ideas will be explored for the development of interfaces (to ion channels and receptors) that enable the real-time (temporal) transduction of molecular (stochastic) events into electrical signals. A critical focus of this program is the exploitation of temporal (kinetic) information for the real-time analysis and detection of molecular targets.

Approach: MOLDICE is a two-phase program addressing the above ideas and concepts. The main goal of the Phase I effort is to demonstrate novel and innovative hybrid bio-molecular device architectures that are scalable to 2D array platforms. Phase I will explore techniques to assemble bio-molecular sensing devices (e.g., engineered protein ion channels/receptors) on a substrate with appropriate interface technologies to couple to silicon circuitry. The Phase I effort will also investigate and demonstrate novel methods to electrically address these bio-molecules at the nanoscale. Issues such as device orientation, spatial distribution, materials compatibility/robustness, signal-to-noise ratio (SNR), ability to scale-up (to arrays), signal bandwidth and temporal resolution, etc. will be addressed during this phase. Technical areas of interest for the Phase I program include:

> **Signal Acquisition and Transduction**: The program will investigate the exploitation of the temporal domain (i.e., kinetics) to develop unique bio-signatures for various molecules of interest to the biological/chemical sensing community. A key focus will be to determine the nature of signals that need to be extracted from hybrid bio-molecular devices and the information content in these signals. The program will explore and develop novel methods to engineer bio-molecular devices to provide specific signals of

interest. Performance metrics for signal acquisition, filtering, amplification and temporal resolution will be quantified for various architectures of hybrid bio-molecular devices.

Electronic Addressability at the Molecular Scale: The program will investigate novel and innovative technologies to enable single molecule addressability at the nanoscale for high SNR transduction of the signals for further processing in silicon. Novel interface technologies will be developed to ultimately convert these signals into real-time electrical signals. The program will explore novel materials and assembly/integration technologies to ensure robust and reliable device operation.

A key Phase I milestone will be the measurement and validation of electrical signals for various target molecules of interest. Phase II will focus on scale-up of the Phase I device architectures into array platforms with large-scale integration and parallelization. An important component of the Phase II effort will be the signal processing task associated with array processing of stochastic signals from individual bio-molecular sensing devices in order to develop bio-signatures. Additionally, Phase II will demonstrate operation and controllability of devices and systems and quantify performance metrics for the array platform (sensitivity, speed, efficiency and ability to automatically distinguish between various biological/chemical species) in the context of various sensing schemes.

This research will lay the foundation for advanced 'biology-to-digital' converter systems that enable direct, real-time conversion of biological signals into digital information. It is anticipated that these developments will lead to new kinds of nanoscale bio-electronic interface technologies extendible to array platforms that enable direct, combinatorial, dynamic (real-time) sampling of stochastic signals from individual bio-molecular receptors in order to develop unique bio-signatures for various target molecules of interest. An important long term deliverable from the program will be a high speed, wide dynamic range Automatic Molecular Recognition (AMR) system for rapid detection, classification and identification of known (and unknown) biological/chemical species.

Anantha Krishnan, Sc. D.
Program Manager, DARPA/DSO

Summaries

Organization: Agave BioSystems

Title: Stabilizing Lipid Membranes with Bacterial S-Layer Proteins

Start Date: December 2002 **End Date:** December 2004

Principal Investigator(s): Joel Tabb
Phone: (607) 272-0002 **Email:** jtabb@agavebio.com
Web: www.agavebio.com

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Project Goals

Bacterial S-Layers are the outermost layer present on the surface of many archeal and bacterial species. These layers are a paracrystalline lattice on nanometer sized pores, often composed of single protein, and all of the information for the self-assembly of S-Layers are contained within the protein. When S-Layers are removed from the cell surface, they will spontaneously reassemble into 2-dimensional sheets at interfaces, such as on lipid bilayer membranes. Extensive studies have shown that S-Layers can significantly increase the stability of these lipid membranes. The goal of this work with respect to the MOLDICE project is to use isolated bacterial S-Layers proteins to stabilize lipid bilayer membranes containing ion channels. Initial studies will be conducted using tip-dip measurements and later studies will be conducted using membranes generated on FET-based electronic devices. Agave BioSystems has also worked on developing preliminary designs for incorporating the FET sensors and S-Layer stabilized lipid membranes into microfluidic modules for toxin testing applications.

Technical Approach

S-Layer proteins from Bacillus sphaericus were extracted from the bacteria, isolated and shown to self-assemble into 2-dimensional lattices. S-Layers were then added to either the electrode or bath side of a tip-dip apparatus and the ability of the S-Layer proteins to stabilize the membranes were measured electronically. Electrophysiological measurements included both the conductance of the lipid bilayer membranes without ion channels and the conductance through ion channels in membranes containing M2 ion channels.

Recent Accomplishments

- S-Layers from Bacillus sphaericus were isolated and were demonstrated to spontaneously reassemble into 2-dimensional lattices.
- Isolated S-Layer proteins were provided to Randy Duran's group of Univ. of Florida, Gainesville for electrophysiological measurements and to Ingo Köper's group at MPI, Mainz, for impedance spectroscopy measurements.
- S-Layer proteins were added to both the electrode and bath side of lipid bilayer membranes and initial experiments showed than S-layers increased the lifetime of the membranes to over 2 hours.
- S-Layer proteins also dramatically increased the physical strength of the lipid bilayer membranes as measured by the ability to plunge the tip-dip membranes deep into the bath solution.
- S-Layers dramatically improved the reproducibility of incorporating active M2 channels into lipid bilayer membranes.
- Electrophysiological measurements demonstrated that S-Layers helped increase the stability of lipid membranes with M2 ion channels from about 20 minutes to over 165 minutes.
- Preliminary microfluidic device designs were developed.

Six-Month Milestones

- Demonstrate that S-Layers can help stabilize ion channel containing membranes for up to 24 hrs and beyond.
- Demonstrate that S-Layers can help stabilize lipid bilayer membranes on electronic sensors.
- Investigate whether chemically crosslinking S-Layer proteins on lipid bilayer membranes will further increase membrane stability.
- Integrate S-Layer proteins into membrane tethers to help stabilize membranes on devices.
- Refine microfluidic device designs for Phase II effort.

Team Member Organization(s)

Henk Keizer and Randy Duran, Department of Chemistry, University of Florida

What Are Bacterial S-Layers?

- **Bacterial surface layer proteins**
- **Coat cell wall of many bacteria**
- **S-Layers are Paracrystalline and are often composed of a single protein**
- **S-Layer proteins contain all of the information for self-assembly**
- **S-Layer proteins have been shown to dramatically stabilize lipid bilayer membranes**

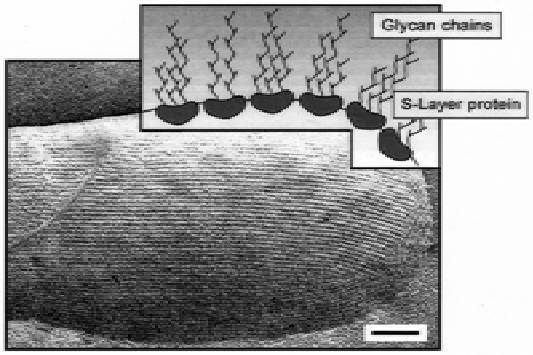

Figure 1. AFM of S-Layer proteins on the surface of *Bacillus sphaericus*. Bar = 100 nm.

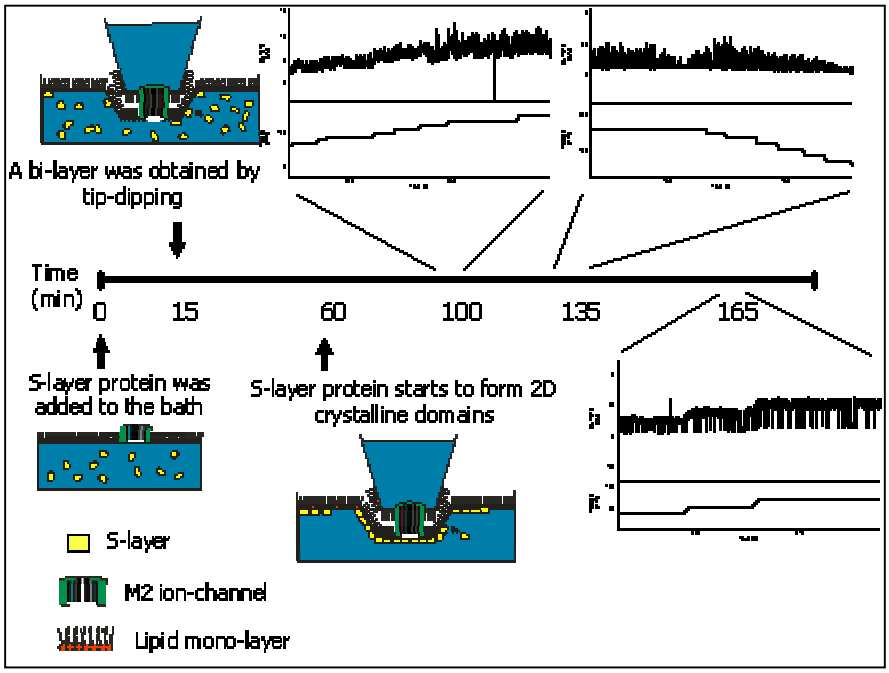

Figure 2. Stabilization of M2 channel activity in tip-dip experiments. An S-Layer protein was added to the bath side of the electrode and electrical recording were taken for 165 min before the membrane was ruptured. Ion channel recordings are shown from 100, 135 and 165 minutes after initiation of the experiment. Without S-Layers, M2 channel recordings typically last 10-15 min and no longer than 30 minutes.

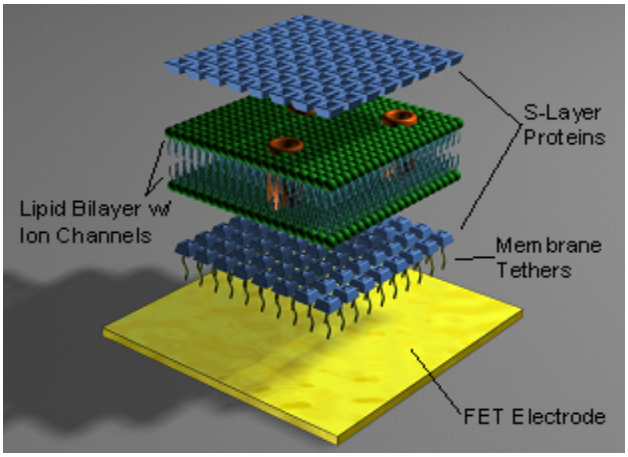

Figure 3. Schematic of how S-Layer proteins could be integrated into lipid bilayer membranes to help stabilize the membranes on FETs. S-Layers can be integrated above the bilayer, below it, or both.

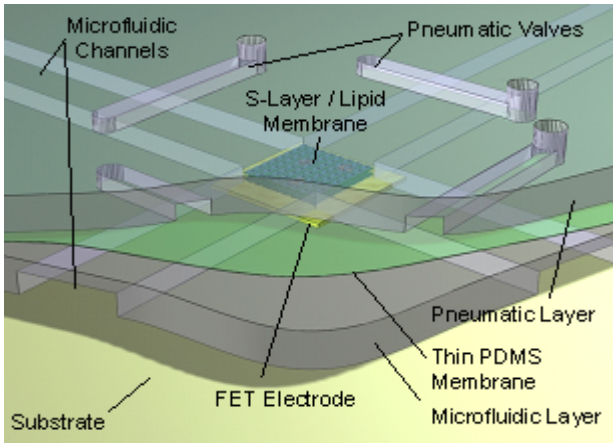

Figure 4. Schematic of one pixel of a proposed multipixel microfluidic device. The S-Layer/Lipid membrane on an FET at the intersection of two fluidic channels. The pneumatic valves control fluid flow in the

Organization: Auburn University

Title: Ion Channel Based Biosensors

Start Date: January 2004 **End Date:** December 2004

Principal Investigator(s): Vitaly Vodyanoy
Phone: (334) 844-5405 **Email:** vodyavi@vetmed.auburn.edu
Web: http://www.vetmed.auburn.edu/index.pl/vodyanoy

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Project Goals

Determine stochastic properties of artificial ion channels on porous support. Stochastic signature of ions of different size and ion blockers of different molecular weights. Determine how chemical modification of ion channels affects the conductive and stochastic properties of artificial ion channels. Stochastic signatures for several classes of target analytes including proteins, nucleic acids, viruses, membrane vesicles and bacteria. Use Langmuir-Blodgett technology and method of molecular assembly to attach specific antibodies, peptides, or phages to the surface of membrane with artificial channels.

Technical Approach

Ion channel based biosensor in which both recognition and transduction are provided by the same molecular devices: the specially designed ion channel. This approach is adapted from biological receptors, which convert chemical signals into currents in ion channels. The biosensors in this work are artificial ion channels constructed by modular design from molecular pores and sensors. The currents through these channels can be registered by conventional methods. The small size and planar architecture of the biosensors allow them to become components of a microelectronic circuit. The biosensors can be used for detection of proteins, toxins, viruses, bacteria, and ions.

Recent Accomplishments

- Optically addressable ion channels on solid substrate. The spatially define generation of a potential gradient applied across membrane was utilized in ion channel based sensors. For open channels, this gradient drives the ion current that is recorded. By clamping the overall membrane at resting potential where ion channels remain closed and show no current activity, a focused beam of light generates a very localized voltage capable of activating one or several channels confined to a small area. Thus, ligand-gated ion channels can be optically addressed and electronically read. By scanning the membrane containing many ion channels, the pattern of activation of the channels can be obtained and the ligand(s) identified.
- Two phages selected from libraries were specific for binding of two proteins, β-galactosidase and streptavidin. These affinity-selected phages can bind their protein antigens, thus functionally mimicking antibodies. Phage biosensors based on QCM device showed high specificity, selectivity and affinity in binding of analytes.
- Phage coat proteins were isolated from phage by using newly introduced "phage skinning" technique. Phage proteins produced stable monolayers on the water/air interface. When phage proteins were reconstituted in phospholipid bilayers they formed discrete and stable ion channels. When channels were formed from the phage selected to bind streptavidin addition of this protein to solution blocks the channel activity.
- A Planar Patch Clamp System with a high resistivity (GOhm) seal has been designed. A silicon substrate (500 μm thick) was used to deposit and pattern a reflowable 500 nm silicon dioxide to form the seal ring. Each seal ring supports a single pore with ~1 μ opening. The structure allows electrical contact and the ability to pull a negative pressure on the membrane helping guarantee a seal and intimate contact with the membrane. The pores can be hardwired or allowed an optical addressing/reading.

Six-Month Milestones

- Fabrication of Planar Silicon Patch Clamp System with a GOhm seal.
- Immobilization of phage derived ion channels in the Planar Silicon Patch Clamp System and analyte detection.

Team Member Organizations

V.A. Petrenko, I.B. Sorokulova; Department of Pathobiology; C.D. Ellis, Department of Electrical Engineering, W.C. Neely, Department of Chemistry, Auburn University, AL, USA

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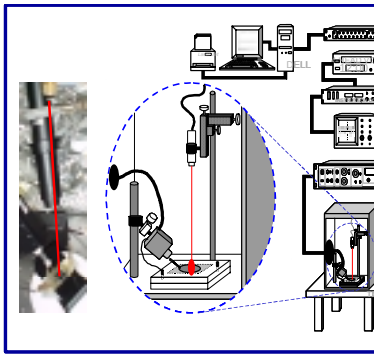
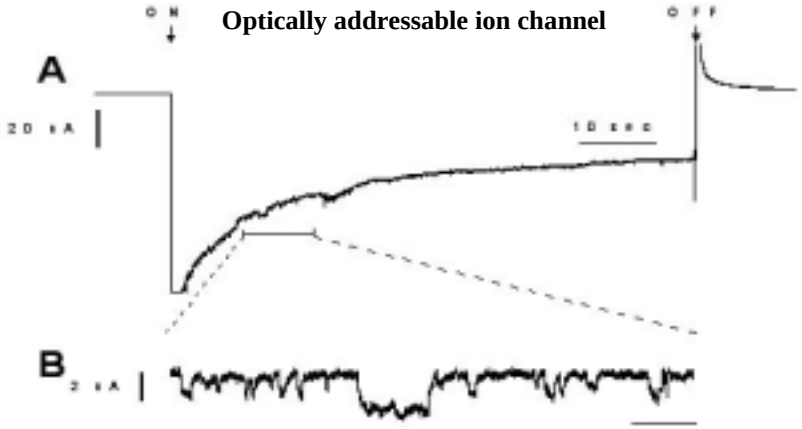


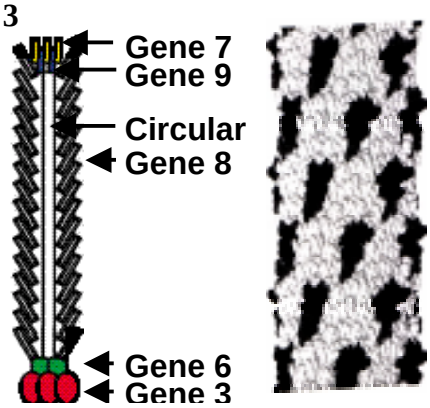
Ion Channel Reader. Laser assembly for performing light scanning experiments on ion channels immobilized in pores of silicon wafers. 0.9 mW 635 nm laser generates the driving force.

2



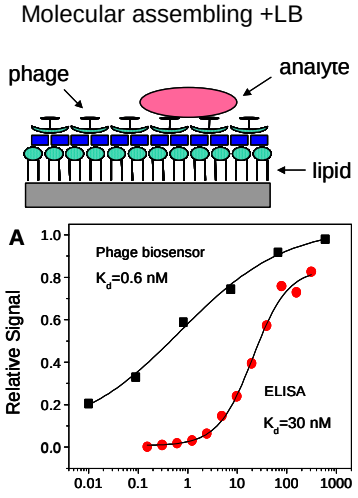
Light generated potential driving ions through amphotericin B channels in lipid membrane coated silicon mesopore wafers (Sandia National Lab). A. Current response due to voltage generated by light. B. An expanded 8 sec segment of the channel current fluctuations.

3



Bacteriophage is a thread-like virus of bacteria (left). It can be modified by genetic engineering methods to introduce new unique structural entities that are displayed on the virion's surface (right).

4



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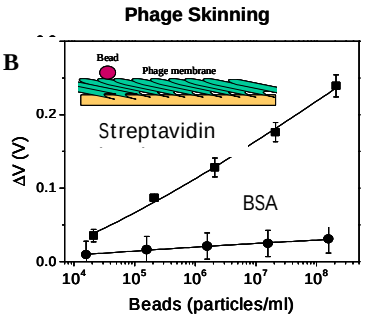

Phage coat QCM sensor for proteins β-galactosidase and streptavidin, fabricated by molecular assembling + LB (A), and phage skinning (B). The phage sensor is rapid, sensitive and specific.

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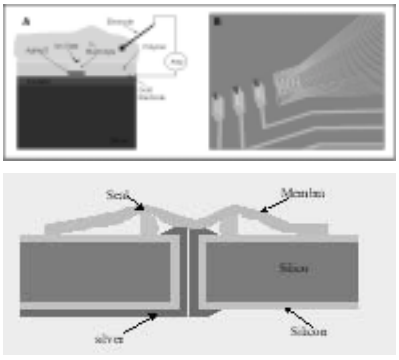

Ion gate membrane electrode array cross-section (A) and arrangement (B) (Upper panel). Planar patch-clamp system allows deposition and voltage-clamp control oreadable ion channels.

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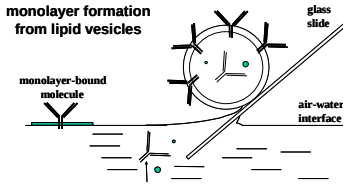
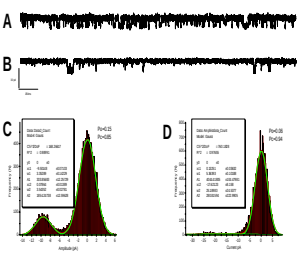


When streptavidin-selective phage proteins were reconstituted in phospholipid bilayers they formed discrete and stable ion channels of uniform size and amplitude of 30 pS (Right, A). The single channels were blocked by the addition of streptavidin resulting in a significant reduction of probability of channel opening (Right, B). The amplitude histograms in the absence and in the presence of streptavidin are shown in panels C and D, respectively.

8 Phage skinning ion channels



Integration of natural and synthetic ion channels with electronic platforms enables rapid, real time conversion of molecular recognition events into digitized electronic signals. Phage Skinning technique provides a target-sensitive and specific ion channels. We employ photoelectrochemical effect for optical addressability.

Optically addressable ion channels on solid substrate. Phage coat proteins were isolated from phage by using new "phage skinning" technique. Phage biosensors based on QCM device showed high specificity, selectivity and affinity in binding analytes. Phage coat proteins formed discrete and stable ion channels controlled by analytes serving as recondition and transducing elements of biosensor.

Organization: Brown University

Title: Direct Nanoscale Conversion of Biomolecular Signals into Electronic Information

Start Date: June 2003 **End Date:** May 2006

DSO DARPA

Principal Investigator(s): Jimmy Xu
Phone: (401) 863-1439 **Email:** 401-863-9107
Web: opto.brown.edu

Project Goals

Direct nanoscale conversion of biomolecular signals into electronic information. Development of a set of innovative bio-nanoelectronic technologies to self-assemble and electrically interface a wide range of proteins and biomolecules with nanoelectronic circuitry. Real-time sensing, control, and actuation of biomolecular reactions and conversion of the bio-signals into digital information

Technical Approach

- Development and demonstration of a novel and scalable protein-nanoelectronic technology platform that enables protein-specific self-assembling via conductive molecular links to nanoelectrodes and arrays.
- Modular development of this platform via protein and peptide engineering, molecular link engineering, and nano electrode development and functionalization.
- Demonstration of this platform in a flow-cell configuration that will consist of a parallel array of multiple protein-electronic sensor units, initially up to 5, but inherently scalable to the level afforded by silicon IC technologies and with ultimate sensitivity and control in the range of tens of molecules per unit.
- Development of new approaches for converting biochemical molecular-recognition events into electronic signals using single conical nanopore membranes and amplification of signal by ionic current.

Recent Accomplishments

- The first site-specific linking of DNA to arrayed carbon nanotubes, and moreover, with appended cargo attached at the other end of the DNA duplex.
- Ultrasensitive DNA detection with nano-disk and nano-wire array electrodes. Sensitivity exceeded 5 attomole.
- Demonstration of individual molecular-scale probe made of carbon nanotubes, functionalized with proteins.
- Site-specific linking of proteins onto carbon nanotube tips and increase of electron transfer rate by >100 times
- Formation of coordinated biomolecular signaling assembly based on the redox enzyme NADH peroxidase.
- Formation of mediated electron transfer system with glycerol 3-phosphate dehydrogenase.
- Formation of self-assembled orientation-controlled photosynthetic RC proteins on functionalized electrodes.
- Demonstration of ten-fold increases of electron transfer from RC to electrodes by controlling RC orientation and by wiring the RC with cytochrome via protein-protein association.
- Prototype fabrication of multi-channel micro-flow chips, with Ag/AgCl reference electrodes, enabling chip-scale cyclic voltammetery.
- Demonstration of single conical nanopore molecular sensors via ionic current changes regulated by molecular recognition events at the pore opening, and of probing of protein-protein interactions via the G-IgG system.

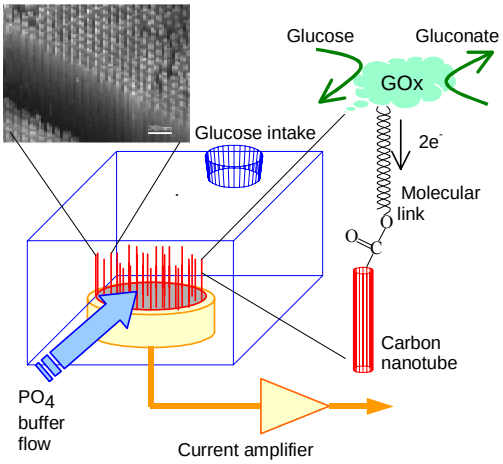
Six-Month Milestones

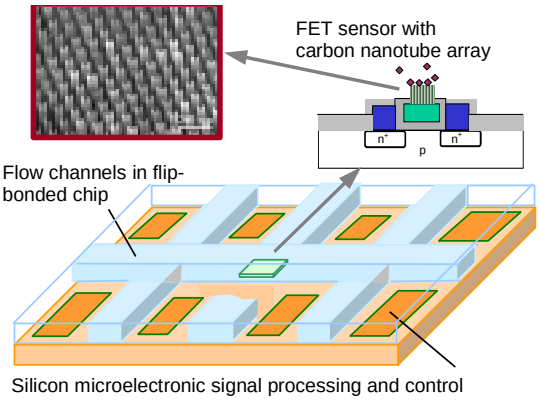
- Extending the DNA immobilization strategies to protein-specific linking to nanoelectrodes.
- Adaptation of the electrocatalytic and ionic detection methods to protein targets, aiming at attomole sensitivity.
- Further development of conductive molecular links by means such as metallization of DNA via substitution bonds or intercalation, engineering of peptides, and quinone linking and LMC construction.
- Further development and optimization of nanoelectrode arrays for efficient electron transfers from proteins.
- Formation of mediated-electron transfer system and characterize the coordinated NADH peroxidase system.

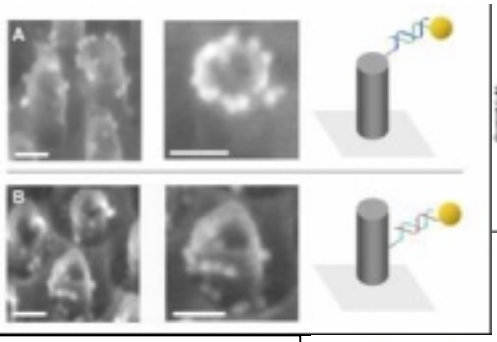
Team Member Organizations

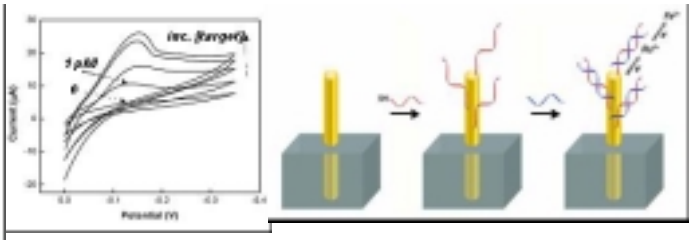
Brown U: J.M. Xu, R. Beresford, J. Yeh; Drexel: I. Chaiken; Boston College: S. Kelley; U Va/NRL: N. Lebedev; U Fl: C. Martin; MIT: S. Zhang

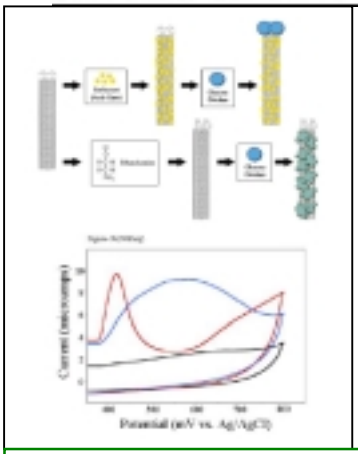
Brown University

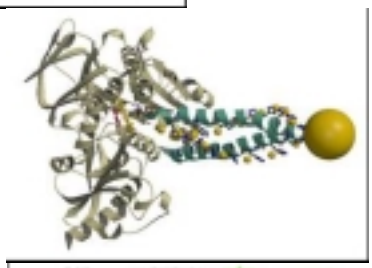


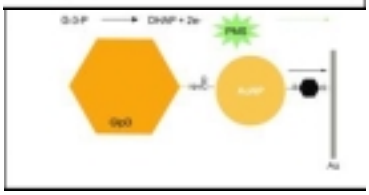


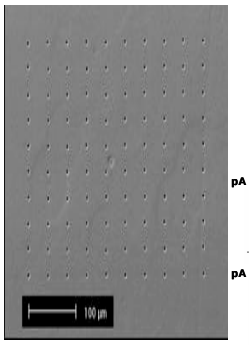


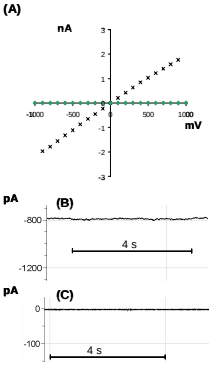


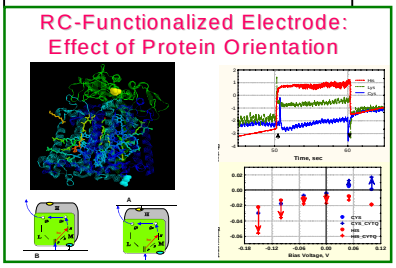


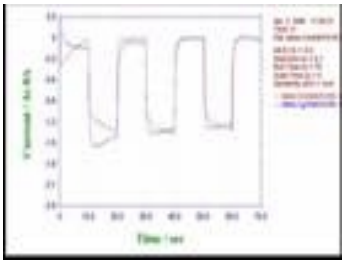


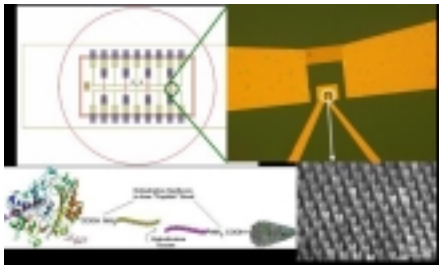












Organization: **Rush Medical Center**

Title: Ionic Channels as Natural Nanodevices

Start Date: December 2002 **End Date:** December 2004

DSO DARPA

Principal Investigator(s): Robert Eisenberg
Phone: 312 942 6467 Email: beisenbe@rush.edu
Web: http://www2.phys.rush.edu/RSEisenberg/physioeis.html

Project Goals

To overcome pitalls in using ion channels in practical devices, specifically
- To learn to maintain stable DC potentials across membranes and channels
- To learn to record stable, artifact-free current through channels in membranes.
- To apply simulants of pathogens to the ompF porin system.

Technical Approach

- ***Understanding how to build a tiny experimental chamber*** that can be replicated to make arrays of addressable devices and can record stable currents free of artifact through individual ion channels.
- ***Understanding electronics and experimental difficulties*** in recording picoamp currents from gigohm devices and in handling lipid bilayers, proteins, and electrochemical cells used to record single channels in bilayers.
- ***Understanding the biology and technology of proteins***. Both biochemical and biophysical approaches are necessary, using the highly developed armamentarium of molecular biology and electrophysiology.
- ***Understanding relevant physical chemistry*** of ions in channels and solution.
- ***Analyzing the effects of pathogen simulants on the ompF/porin system***. Studying simulants is absolutely necessary if practical devices are to be made available for military use.

Recent Accomplishments

- A lipid bilayer containing the voltage activated K channel and porin have been implemented in Langevin Poisson and transport simulations in three dimensions to allow validation of the setup and its properties.
- Silicon wafer has been constructed with deep etch processing optimized to allow deep etch profile and sidewall roughness to allow satisfactory attachment of lipid bilayers to the silicon substrate.
- Bilayers have been formed on silicon surfaces modified with PTFE (Teflon) with minimal shunt resistance, i.e., in a normal manner.
- Channels have been inserted into bilayers on the PTFE coated silicon and recordings have been made that are indistinguishable from those on conventional setups.
- Planar Ag|AgCl electrodes were fabricated repeatedly and routinely and their electrochemical stability was assessed. Electrodes show low drift (~1 mv) over a time period of hours.
- An integrated device include PTFE coated surfaces, isolated silicon (for low capacitance and low noise) and planar Ag|AgCl electrodes has been buil. Bilayers have been made on a protoptye with gigaseal resistance.
- **Single molecules of porin have been inserted into a prototype of the channels-on-silicon COS integrated device** and recordings of single channels have been made that are indistinguishable from those on conventional setups. **This nearly represents the completion of the main goal of phase 1 of our project.** As far as we know, no one has recorded single channels in set up built formed on silicon substrates suitable for integrated circuit construction using standard techniques

Six-Month Milestones

- To fabricate **COS** in substantial numbers available to other contractors in Moldice.
- To incorporate devices for control of gigaseals into the integrated **COS** device
- To reconstitute and record from single channels of hemolysin instead of porin in the integrated **COS** device

Team Member Organization(s)

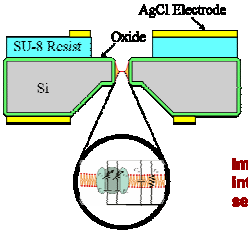
Illinois Institute of Technology, Arizona State University, University of Illinois Urbana Champaign

Project Goals

Goal 1: Embed channels in an integrated device that maintains stable potential across them and allows recording of stable, artifact free current through them.

Goal 2: Find simulants that bind and transiently block conduction of ions through OmpF.*

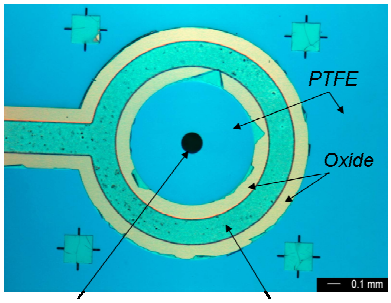
** we shall work with DARPA and other groups within the MOLDICE network to incorporate ion channels that show desired properties*



Important building blocks of a fully integrated biosensor with on-chip sensing and signal processing

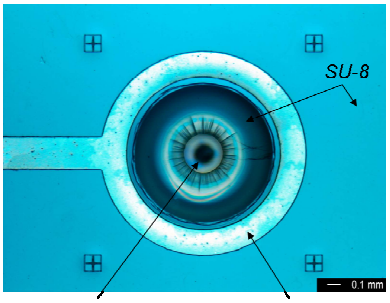
Integrated AgCl Electrodes

Silver electrode on oxide



- the PTFE layer is removed from the electrodes using lift-off

Silver electrode on SU-8

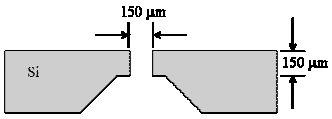


- 800 nm of silver is evaporated on both sides of the wafer

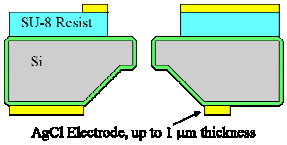
Process Flow

Resist for Initial Hole Etching

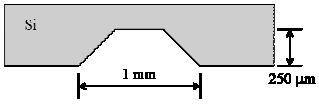
Small Hole Etching



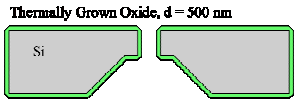
AgCl Electrode



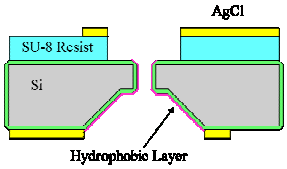
Large Hole Etching



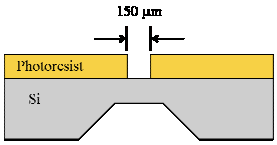
Thermal Oxidation



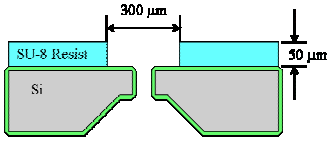
Surface Modification Layer



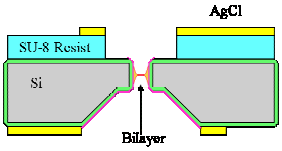
Resist for Small Hole Etching



SU-8 Resist (Epoxy)

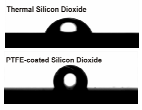


Lipid Bilayer Attachment

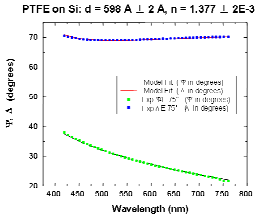


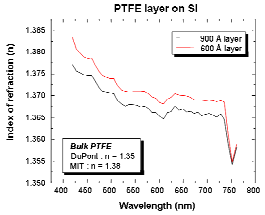
PTFE Surface Modification

- the stability of the lipid bilayer is related to the contact angle between the bilayer and the supporting substrate
- water contact angle measurements can be used to determine the substrate's surface energy



- coating the oxide surface with a Teflon film changes its properties from hydrophilic to hydrophobic (small to large contact angle)
- Plasma CVD provides an easy way of depositing several nm thick PTFE layers





- good agreement between model and experimental ellipsometric data allows a reliable thickness measurement
- dispersion curve indicates a high density PTFE polymer layer similar to bulk material
- "stackable" layers

Plasma CVD is an interesting novel method to provide PTFE surface modification layers for lipid bilayer attachment to solid supports

DSO DARPA

Organization: University of California, Los Angeles

Title: Integrated Massively Parallel Arrays of Stochastic Sensors (IMPASS)

Start Date: July 2003 **End Date:** December 2004

Principal Investigator(s): Carlo Montemagno/Jacob Schmidt
Phone: (310) 794-7270/(310) 825-4451
Email: cdm@seas.ucla.edu/ schmidt@seas.ucla.edu
Web: www.bioeng.ucla.edu

Project Goals

The overall goal is the development of a technological foundation for synthesis of protein/polymer hybrid membrane sensors. We aim to create biomimetic membranes which are more robust and longer-lived than their natural lipid counterparts. These membranes will house membrane proteins which are molecular sensors and transporters in nature; these membranes will be able to house any membrane protein selected, enabling a large variety of possible composites. Further work will focus on the creation of large area membranes and interfacing these membranes with external devices. Microfabricated substrates will allow device miniaturization as well as parallel addressability of sensors.

Technical Approach

Over the past six months, we have concentrated on measuring polymer membrane lifetime and incorporating protein into the polymer membranes. We measured the polymer on teflon supports as well as one microfabricated substrates. We varied the surface chemistry in a controlled manner. We incorporated alamethicin and OmpG into the polymer membranes in order to learn about the probability of protein incorporation, and the effects of the polymer membrane on the incorporated protein. We have also collaborated with Stephen Cheley from Texas A&M, who has donated α-hemolysin, which we have also incorporated into polymer membranes. We have also donated our polymer to Trevor Thorton and coworkers for use with their microfabricated substrates.

Recent Accomplishments

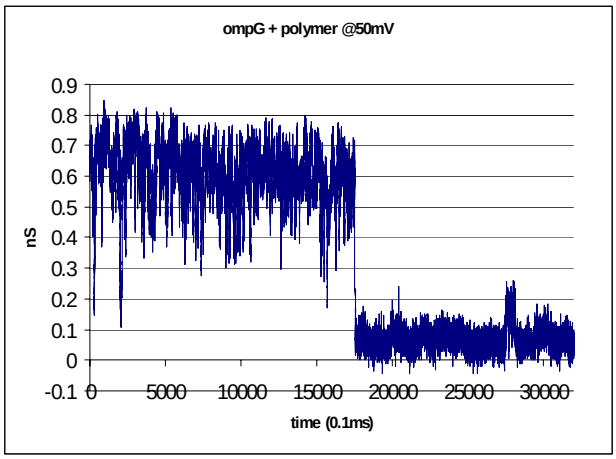
- We have measured the conductance of OmpG in polymer membranes.
- We have measured the conductance of α-hemolysin in polymer membranes.
- We have measured the mean lifetime of the polymer membranes.

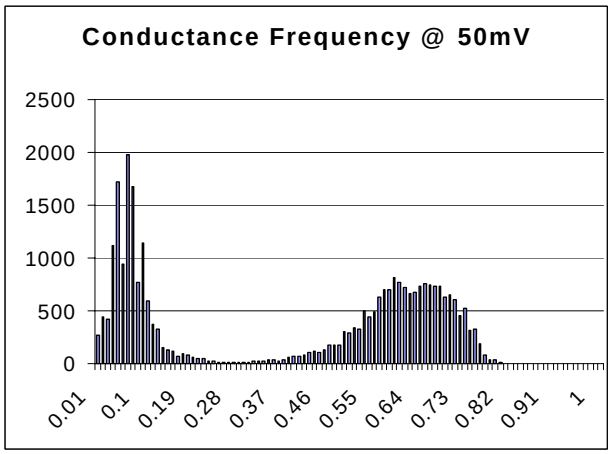
Six-Month Milestones

- Create composite membranes of incorporated protein
- Measure gated transport of protein/polymer membranes

Team Member Organizations

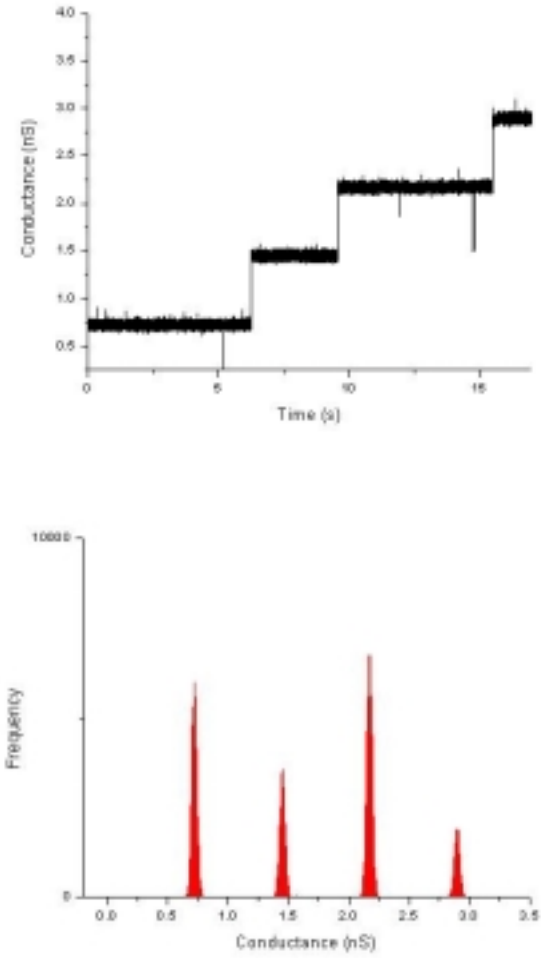
Department of Bioengineering, UCLA





Top: Conductance versus time for OmpG incorporated into a polymer membrane. The protein closes halfway through the recording. Applied voltage = 50 mV.

Bottom: Histogram of the data in the top graph. The higher peak shows that OmpG has a mean conductance of ~.7nS, showing the effects of the increased polymer membrane stiffness and stability on the protein conductance.



Incorporation of multiple α-hemolysin into a polymer membrane. Top: Transport measurements following addition of α-hemolysin to the solution surrounding the membrane show the stepwise incorporation of 4 proteins. One protein is incorporated at the beginning of the trace, and three more incorporate shortly thereafter. **Bottom:** Histogram data of the top showing the same .72 nS conductance for each protein.

DSO DARPA

Organization: University of Florida

Title: Addressable Immobilized Ion Channels

Start Date: July 2003 **End Date:** December 2004

Principal Investigator(s): Randy Duran
Phone: (352) 392-2011 **Email:** duran@chem.ufl.edu
Web: www.chem.ufl.edu/~duran/DARPA

Project Goals

We will engineer films of oriented, addressable, ion channels by surface immobilization and modification with stabilizing overcoatings. We will incorporate one of a logically chosen sequence of four ion channel systems with ion sensitive field effect transistors (ISFET's), or microelectrode arrays (MEA's), by general methods, not requiring intricate chemical modification of each biomolecule system. We will address key enabling research results to optimize the transition to Phase 2 studies, where thin film/chip array geometry assemblies will be integrated with mixed populations of different ion channels. We also seek to achieve bilayer membrane resistances > 10^6 Ω-cm^2, quantifying response times, and measuring single channel current fluctuations of the ion channels.

Technical Approach

We will incorporate functional ion channels into membrane bilayers tethered to ISFET and MEA devices or microelectrodes as extended gates of FET's. We will measure single channel current fluctuations and other stochastic signals associated with these ion channels. These devices will be stabilized such that they retain enhanced performance in fluctuating environmental conditions compared to "bare" membrane bilayers. Agonist/antagonist as well as voltage gating effects will be examined.

Recent Accomplishments

- Production of a library of genetically engineered Maxi-K mutants and 2 point mutants of M2.
- Extended (hours) single channel recordings on s-layer stabilized ion channel assemblies by Tip Dip Electrophysiology.
- Demonstrated measurement ability of Si-based microelectronics with simulated single channel currents.
- Teaming to include China Lake, Agave Biosystems, Electronic BioSciences, and University of Miami for s-layer, systems integration, and signal analysis.

Six-Month Milestones

- to establish tight lipid bilayers on FET device.
- to measure pharmacological single channel response.
- to obtain single channel stochastic current from Maxi-K mutants.
- to demonstrate enhanced (days) stability of ion channel/lipid assemblies during measurement.

Team Member Organization(s)

University of Florida : Chemistry Department, McKnight Brain Institute, Whitney Laboratory; *Max Planck Institute for Polymer Research*, NRL, UT-Austin, Agave Biosystems, Electronic BioSciences, Univ. of Miami

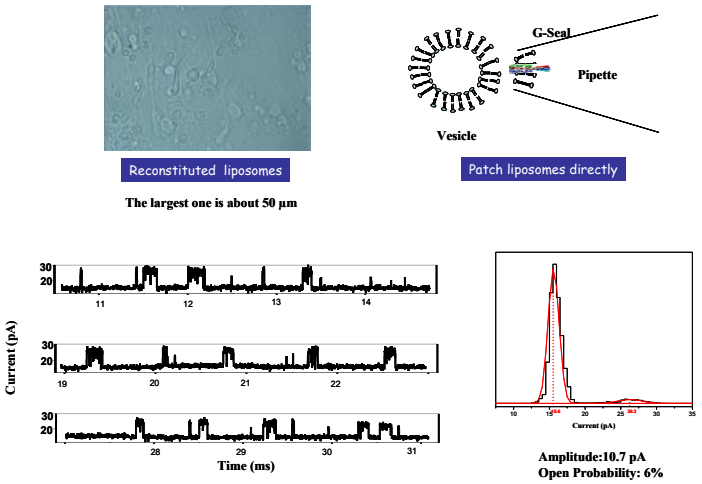


Figure 1: We have reconstituted liposomes of Maxi-K containing ion channels from natural lipid to more stable phytanyl-based lipids and obtained single channel fluctuations directly from these

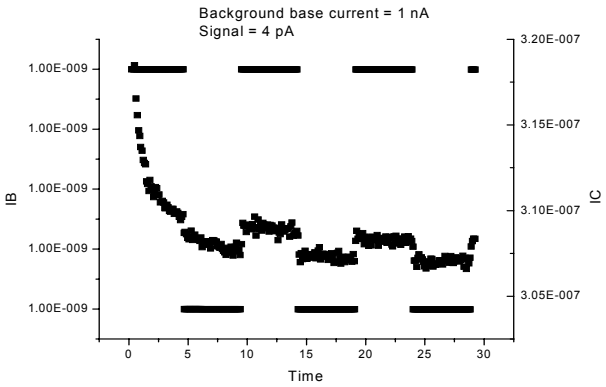


Figure 3: Using single channel current data obtained at Florida, our collaborators at Texas have tested the response of a bipolar transistor design as a potential Si-based sensing element. 4 picoamp fluctuations observed in a real channel are easily measured

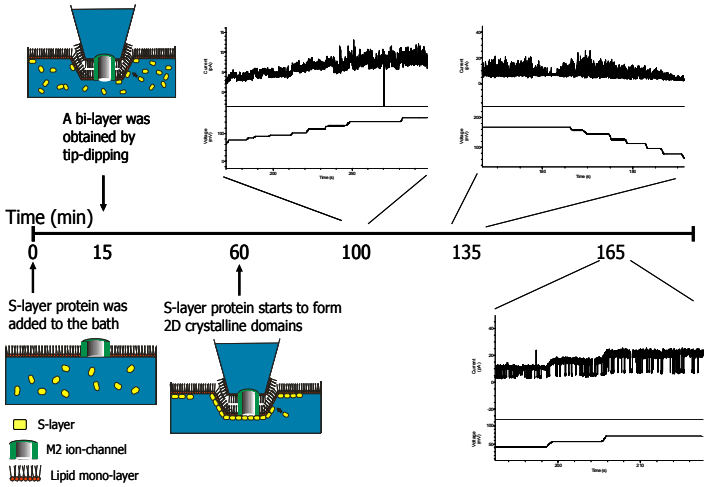


Figure 2: We have achieved routine multi-hour single channel fluctuation signals via stabilizing M2 channels with an s-layer protein overlayer

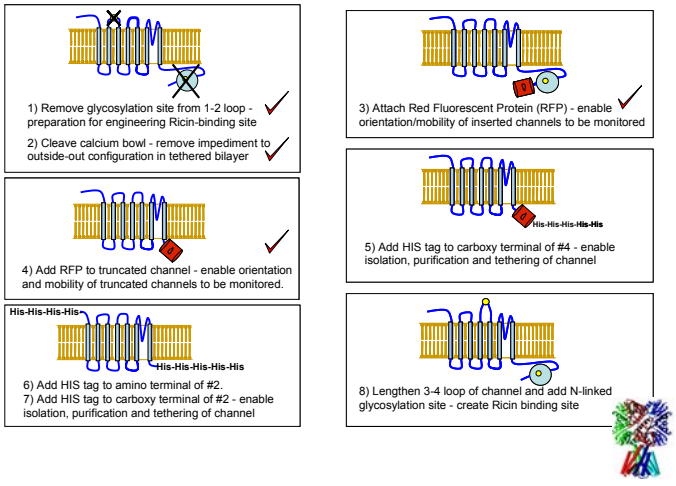


Figure 4: We have engineered a library of Maxi-K mutants designed to broaden the overall stochastic sensitivity of our devices and to enhance Ricin sensitivity

DSO DARPA

Organization: University of Florida

Title: Array-Based Nanopore Stochastic Sensors for Multiplexed Bioassays

Start Date: July 2003 **End Date:** December 2004

Principal Investigator(s): Charles R. Martin
Phone: (352) 392-8205 **Email:** crmartin@chem.ufl.edu
Web: http://www.chem.ufl.edu/~crmartin/

Project Goals

We are developing array-based nanopore stochastic sensors that allow for the simultaneous analysis of numerous different classes of analyte species with a single rugged and compact sensing platform. Our immediate efforts have been focused on single-element sensors of this type.

Technical Approach

The array-based nanopore stochastic sensors build on the work of Prof. Hagan Bayley on protein-channel based stochastic sensors and the work of Prof. Charles Martin on synthetic nanopore membranes. The sensors consist of a synthetic membrane containing an array of conically shaped nanopores arranged on a regular square lattice. In one embodiment of this concept, an engineered protein channel (e.g., α-hemolysin) is inserted into the mouth of each nanopore, and each nanopore with its inserted channel constitutes a sensing element in this array-based sensor. We call this sensor-design #1. In a second embodiment (sensor-design #2), building on the work of Prof. Paul Cremer, the protein channels are immobilized in a supported bilayer membrane coating the face of the nanopore membrane. In addition, to these protein channel-based strategies, analogous totally abiotic array-based nanopore stochastic sensors are being developed (sensor design #3).
For both the protein channel and abiotic devices, a common electrode will be used to pass an ionic current through each of the sensing elements. In the ultimate embodiment of this concept, the individual ion currents carried by each of the channels will be measured using an array of microtip electrodes configured in the same lattice pattern as the nanopores. These stochastically fluctuating ion currents will provide both the chemical identity and the concentration of the analyte. Prof. Henry White has extensive experience in using microtip electrodes to study currents in nanopores. Prof. White's experience in modeling such systems is also invaluable to the research effort.

Recent Accomplishments

We have preliminary data that suggest that both sensor design #1 and #2 have been successfully achieved. We have a large amount of experimental data on sensor design #3. This abiotic approach to stochastic sensing is, in fact, much easier.

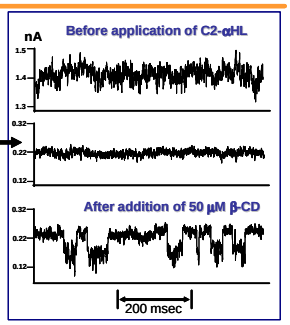
Six-Month Milestones

We must reproduce the data we presented at the DC meeting in August '04 that showed that we had functioning sensor designs #1 and #2.

Team Member Organizations

Professor Charles Martin, Department of Chemistry and Center for Research at the Bio/Nano Interface, University of Florida; Professor Hagan Bayley, Department of Chemistry, Oxford University; Professor Henry S. White, Department of Chemistry, University of Utah; Professor Paul S. Cremer, Department of Chemistry, Texas A&M University

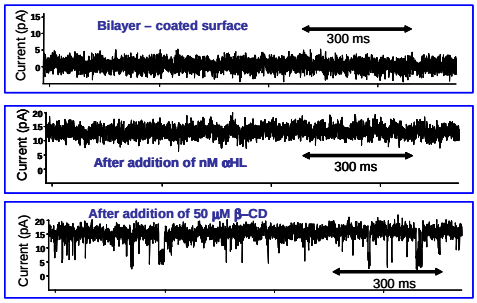
Preliminary Stochastic Sensing Data from a Sensor-Design # 1 Device*



Key point - unlike analogous data with wt-αHL, the higher current state cannot be obtained by reversing the polarity; i.e. immobilization is permanent.

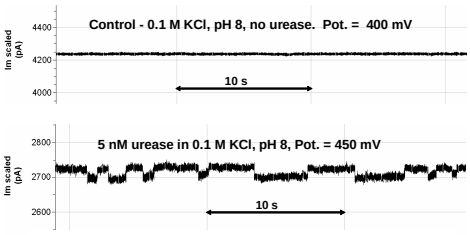
*C2-αHL mutant immobilized in a gold-coated nanopore membrane. Transmembrane potential = 1 V.

Preliminary Stochastic Sensing Data from a Sensor-Design # 2 Device*



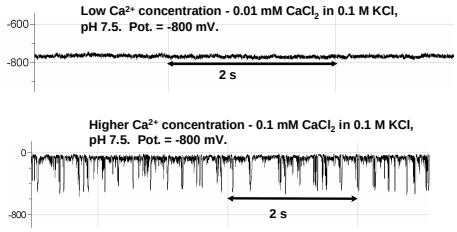
*Single nanopore Kapton. Transmembrane pot.= 1 V. Pore openings = 4 nm and 1.25 μm.

Abiotic Stochastic Sensing of the Protein Urease*



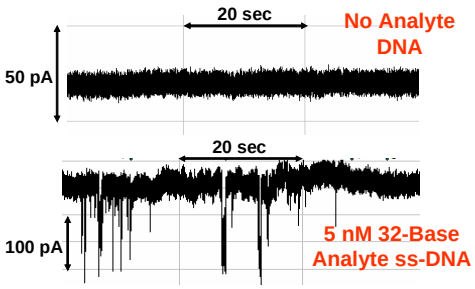
*Single gold nanotube, openings = 5 microns and 40 nm.

Abiotic Stochastic Sensing of Ca^{2+}*



*PET conical nanopore. No gold. Pore openings ~600 nm and ~5 nm

Abiotic Stochastic Sensing of ssDNA*



Organization: University of Illinois

Title: Single Molecule Detection Using a Silicon Nanopore-Nanotransistor IC

Start Date: April 2004 **End Date:** March 2005

Principal Investigator(s): Gregory Timp
Phone: (217) 244 9629 **Email:** gtimp@uiuc.edu

DSO DARPA

Project Goals

We plan to produce, test and simulate the performance of 1-10nm diameter pores through 10-65 nanometer-thick membranes fabricated from MOS-capacitors to explore their use for sequencing a single DNA molecule.

Technical Approach

To determine the chemical sequence of a single DNA molecule, we intend to measure the dipole moment induced in the capacitor by each nucleotide as the molecule is electrophoretically driven across the membrane through the nanopore. We also plan a hierarchy of simulations and measurements, in concert, to establish the accuracy of MD simulations of nanopores, which will be used to predict the performance and optimize the sensor design. First, the ionic conductivity through the pore will be measured and compared with the results of multi-scale simulations that are used to compute I-V characteristics, using molecular dynamic (MD) simulations to account for the charge in the pore, the structure and viscosity of the water, and reduced ion mobility. Second, we will accomplish simulations and measurements of permeation of nanopores by so-called channel blockers, organic ions that block biological membrane channels. And third, simulations and experiments will focus on the translocation of short *DNA* <100-mer through the nanopore.

Recent Accomplishments

- Working in conjunction with the New Jersey Nanotechnology Consortium (NJNC), we have produced membranes in Si_3N_4, *Si* and *poly-Si/*SiO_2*/Si* ranging in thickness from 10-65nm thick (see Figure 1). And using electron beam-induced sputtering, we have fabricated nanometer-diameter pores in these membranes.
- We have measured the electrolytic transport through the nanopores produced in Si_3N_4 and *Si*, *poly-Si/*SiO_2*/Si* membranes in *KCl* as a function of concentration from 0.01M to 1M, and simulated the transport using molecular dynamics (MD). As illustrated by Fig. 2, to account for the measured conductivity observed in different sized nanopores, we investigated the effects of: (1) pore surface atoms that carry no polarization partial charge and zero net surface charge, (2) pore surface atoms that carry no polarization partial charge, but has a net surface charge, (3) pore surface atoms carry polarization partial charges but zero net surface charge, and (4) pore surface atoms carry polarization partial charges. When polarization partial charges on the surface atoms are considered, the molecular dynamics simulations show a better agreement with the experimental data especially for 1nm diameter pores, but there is still disagreement between MD and the measurements for larger diameters.
- We also observed evidence of the translocation of single *DNA* molecules through nanopores produced in Si_3N_4 and *Si*, *poly-Si/*SiO_2*/Si* membranes in the electrolytic current through the pore, and corroborated the translocation events using quantitative PCR and MD. When the *DNA* is electrophoretically driven into the pore, the ion current is blocked, resulting in a transient in the electrolytic current through pore. Through experiment and MD, we have extensively characterized the blocking-current transients as a function of the geometry of the pore (see Fig. 2). MD predicts especially stringent specifications for the pore diameter required to maximize the dipole moment induced in the capacitor membrane during a translocation (see Fig. 3.) We established through experiments that single-stranded *DNA* can translocate through diameter pores <1.4nm in diameter under certain electric fields conditions, which is the optimum diameter for detection of the moment according to MD. Finally, for the first time, we have detected the voltage signal associated with a single molecule of DNA translocating through a 2.5nm diameter pore in a heavily doped *Si*-membrane electrode. The ~10mV signal corresponds precisely with the duration of the current blockade. This signal level is consistent with the prediction of a self-consistent solution to Poisson's equation associated with the *DNA* translocation accomplished using snapshots of the molecular dynamics simulations to specify the position of the molecule as illustrated in Fig. 4.

Team Member Organization(s)

The UIUC team consists of: Klaus Schulten, Jean-Pierre Leburton and Narayan Aluru.
The NJNC team consists of: Avi Kornblit, Fred Klemens, Tom Sorsch and John Miner

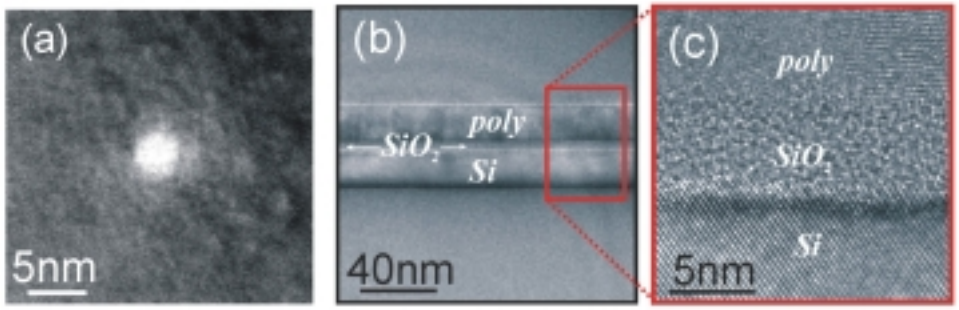


Figure 1. A Scanning Transmission Electron Micrograph of a nanopore (a) sputtered using a tightly focused, high -energy electron beam into a MOS-cap membrane (b) comprised of a SiO_2 film about 5nm thick as shown in (c) sandwiched between two heavily doped silicon layers each about 20nm thick. The polysilicon and silicon electrodes will be used to detect the induced voltage as a DNA molecule translocated across the membrane through the nanop

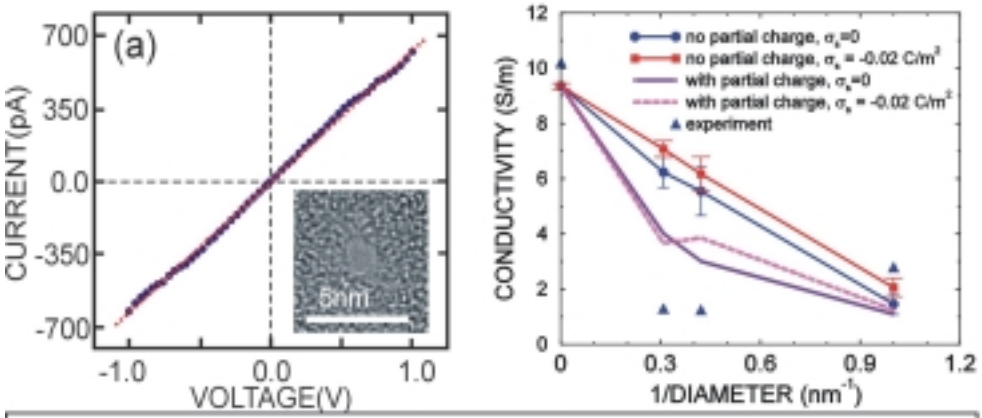


Figure 2. (a) The electrolytic current measured in 1*MKCl* through a 1nm diameter nanopore in a Si_3N_4 membrane ~10nm thick. The conductance is practically linear over ±1V range. We infer the conductivity from the geometry of the pore estimated from TEM and the conductance measured at 100mV. (b) The conductivity of pores in Si_3N_4 as calculated by MD using four different approaches. Notice that calculations that include the effect of partial charge approach the measurements.

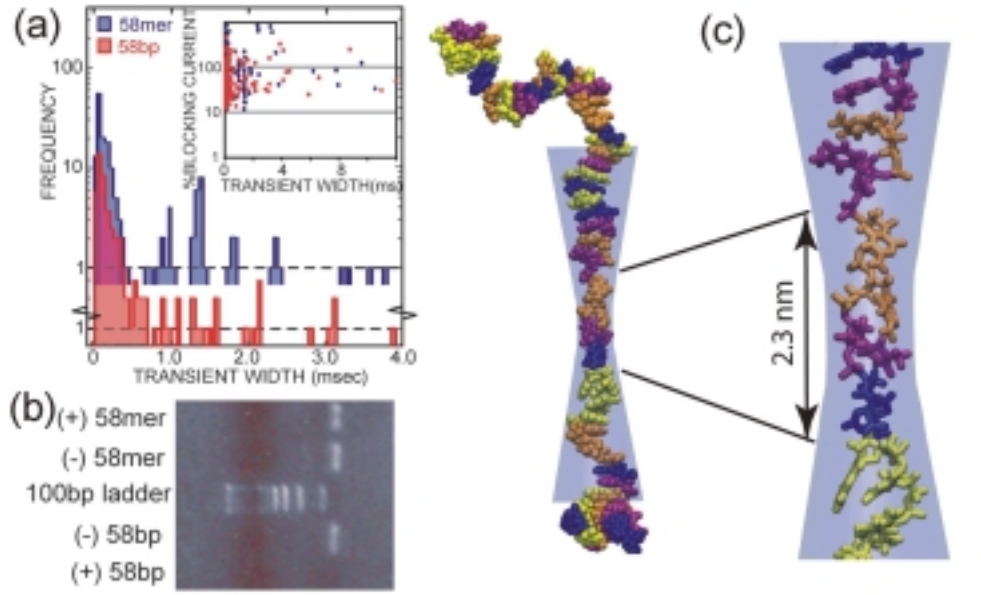


Figure 3. (a) A histogram of the duration of current blockade transients observed when 58 -mer single stranded DNA and 58 bp double stranded DNA interact with a 1nm diameter pore. Notice that the long duration events are observed pred ominately for ssDNA. Using PCR and gel electrophoresis (b), we find indications of both single and double stranded DNA at the negative electrode, but only single stranded DNA at the positive electrode. Thus, only ssDNA translocates across the membrane through the pore. (c) MD simulation of the m icroscopic conformation of a 58 -nucleotide DNA strand placed inside a 1.0 -nm-diameter pore. At the start of the simulation, a 58-nucleotide DNA strand floating in a 1M water solution of KCl was enclosed by a surface rep resenting a 2.0 -nm-diameter pore. A 10 pN force pointing towards the pore center was applied to every DNA atom positioned outside of the pore surface. In a 1.5 ns simulation the radius of the pore was gradually reduced to 1.0 nm. Geometrical restrains indu ced changes in the DNA conformation: nucleotide bases aligned with the pore axis, the distance between consecutive nucleotides increased from 0.35 nm of unrestrained DNA to 0.7 nm. Thus, a 1.0 -nm-diameter pore in a 2.0 -nm-thick membrane can accommodate onl y 3 nucleotides. Water was found to be partially excluded from the narrowest part of the pore. Both DNA stretching and partial water exclusion are expected to result in a stronger sequence-dependent signal if compared to the signal from an unrestrained DNA.

DSO DARPA

Organization: University of Massachusetts, Amherst

Title: Molecular Modeling of Stochastic Sensing of Biomolecules

Start Date: January 2004 **End Date:** December 2004

Principal Investigator(s): M. Muthukumar
Phone: (413) 577-1212 **Email:** muthu@polysci.umass.edu
Web: http://theory.pse.umass.edu

Project Goals

- To establish the molecular mechanism of the stochastic sensing (SS).
- Determination of optimum values of various control parameters in SS of biomolecules by the alpha-hemolysin pore, using molecular modeling.
- Simulation of particle sensing by alpha-hemolysin pore, using the Langevin dynamics simulations.

Technical Approach

The molecular modeling program used in the current investigations involves a self-consistent computation of the coordinates of analytes (employing the Langevin dynamics algorithm) and the ionic current of the alpha-hemolysin channel (using the modified Poisson-Nernst-Planck formalism), during the process of SS.

Recent Accomplishments

- By modeling the ionic current signature of SS of analytes by the alpha-hemolysin pore containing tethers, we have discovered the molecular details of SS, in terms of the specificity of the analyte and tether characteristics (chemistry, location of anchoring, and length).
- We have modeled the ionic current characteristics of the alpha-hemolysin channel as charged particles get trapped electrophoretically at the mouth of the vestibule, in terms of the size and charge of the particle. This paves the way to design sensing/sorting particles.

Six-Month Milestones

- Validation of modeling results with respect to experimental results on SS
- Implementation of concepts from the modeling in the design of the tether molecules and their location for an efficient detection of biomolecules
- Development of multiscale modeling to scale up the detection of large particles and organisms
- Development of optimized arrays for SS

Team Member Organizations

University of Massachusetts, Amherst

Figure 1. Two situations of SS by alpha-hemolysin pore and their corresponding ionic current traces.

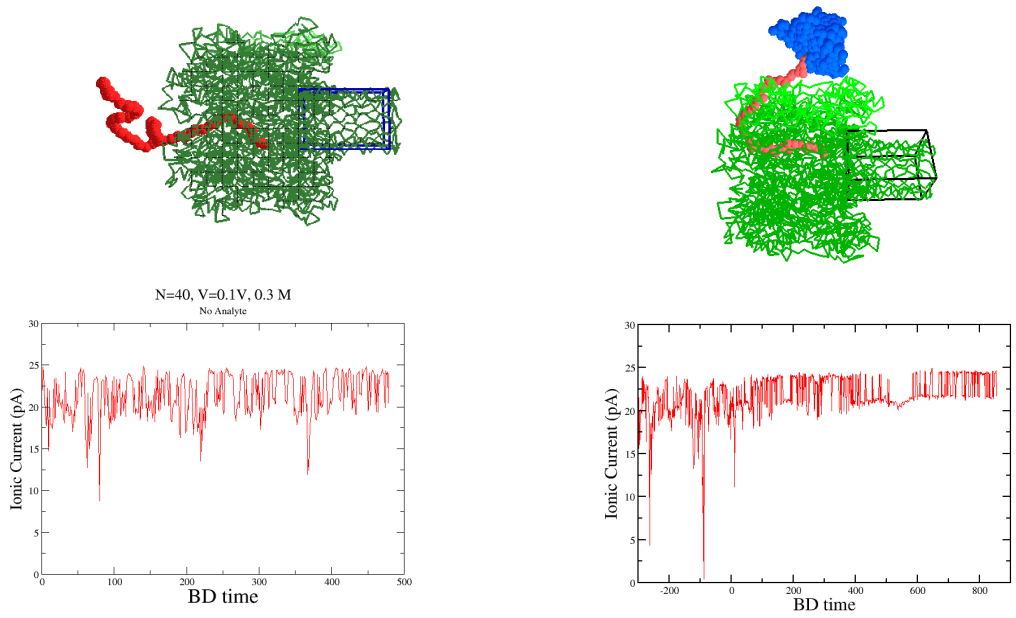
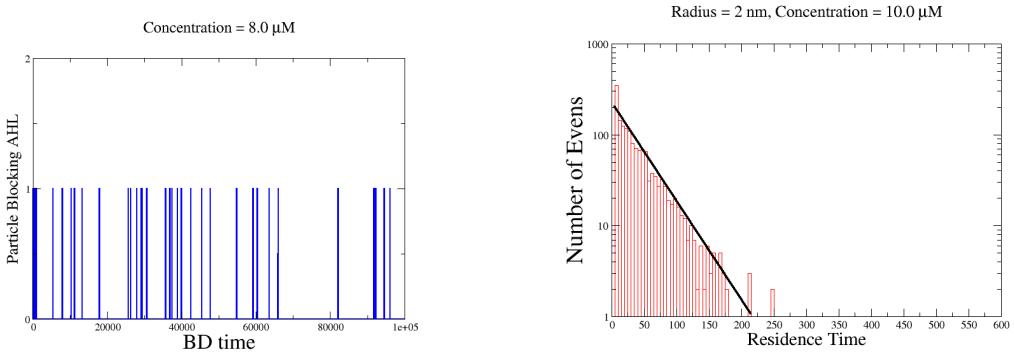


Figure 2. Design of particle-sensing by SS. (a) Typical histogram of pore blockades. (b) Typical histogram of residence time.



(a) (b)

DSO DARPA

Organization: University of Texas

Title: Novel Devices for Biosensing

Start Date: March 2004 **End Date:** February 2005

Principal Investigator(s): Ananth Dodabalapur
Phone: (512) 232-1890 **Email:** ananth@mail.utexas.edu

Project Goals

The goals of this project are to create detection and amplification technologies for ion current sensing. These technologies must also be easy to integrate with biological materials. We are pursuing two strategies: the first is the use of silicon bipolar technology (designed to have high current gains at low currents) that are expected to be ideally suited for this function. Bipolar transistors are natural current amplifiers and can possess high gain. They can be used in circuits (for Phase II) that amplify the signal at the source and facilitate signal processing. The second strategy is using constricted channel FET technology.

Technical Approach

We are using silicon epi wafers to fabricate bipolar transistors, which will possess a gain of > 100 at low collector currents. We are also developing a process that enables the integration of such devices to tethering layers, lipid bilayers, and ion channels, while retaining the good electrical characteristics. The noise properties of these devices will be evaluated. We will work with team members, particularly U. of Florida (Duran) and Max Planck Institute (Knoll) to understand interfacing issues with biomaterials.

Recent Accomplishments

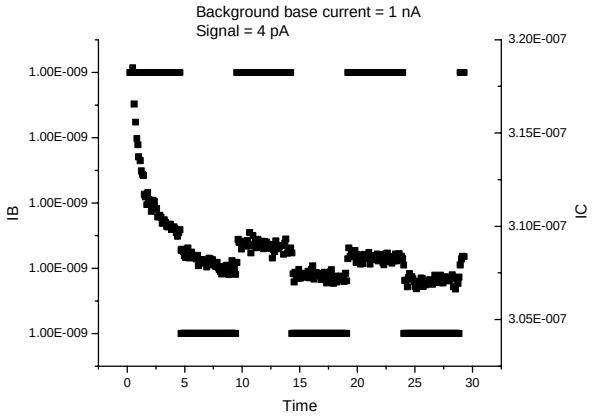
- Design of high current gain bipolar transistor
- Verification of design with MEDICI
- Demonstration of amplification of ~ pA level current with a BJT with a current gain of > 2000
- Evaluation of Au-Pd alloys for smooth interfaces for SAM binding
- A commercially available bipolar transistor was used in this experiment as a prelude to using ones fabricated at UT. In this experiment the bipolar which was chosen so as to have a high current gain at low collector currents was biased with a DC base current of

Six-Month Milestones

- Demonstration of high gain BJTs based upon our design
- Noise properties of BJTs in an ion rich ambient
- Interfacing with ion channels
- Fabrication of FET devices

Team Member Organizations

Randy Duran (University of Florida)



1 nA. A time-varying signal current of magnitude 2 pA – 5 pA was superposed on this current. This current mimics the ion-channel current. The collector current modulation is clearly seen in the figure above, which is for a signal current of 4 pA. The magnitude of the magnitude is ~ 1 nA, clearly demonstrating that detection of low currents is possible at a gain of > 200. This approach will be reproduced in integrated devices where actual ion-channel currents will be detected and amplified. The bandwidth was limited by measurement instrumentation limitations.

Organization: University of Wisconsin

Title: Single Receptor Interfaces for Real-Time Kinetics

Start Date: June 2003 **End Date:** May 2006

Principal Investigator(s): Daniel van der Weide/Robert Blick
Phone: (608)-265-6561/(608)-262-1954
Email:danvdw@engr.wisc.edu/blick@engr.wisc.edu
Web: http://lmse.engr.wisc.edu

DSO DARPA

Project Goals

- To convert receptor binding kinetics into real-time electrical signals using temporal resolution in the nanosecond regime
- Nanofabricate apertures in low-capacitance glass to support bilayer membranes that can be interfaced to planar microwave circuits
- Develop arrays of microwave/nanoscale interfaces with appropriate readout circuitry

Technical Approach

We propose a fundamentally new approach to this interface: a nanoscale microwave probe measuring both reflection and transmission through an artificial (supported) membrane having a single receptor embedded into it. While this is a fundamental departure from conventional patch-clamp recording on black lipid (BLM) or cell membranes, we have achieved initial demonstrations of the idea. The 0.001-100 MHz bandwidth of a microwave receiver, combined with its inherent immunity to environmental noise, offers important capabilities in solving the speed vs. resolution puzzle and thus achieving the goals of the MOLDICE program. In this proposal we will implement the receptor (initially a porin, but later a GPCR or ion channel), BLM and readout circuitry in a silicon and SiO_2 environment with microwave readout using modified circuits developed for wireless communications to maximize our success and the ultimate flexibility of implementation and ultimately, remote query.

Recent Accomplishments

- Achieved the first simultaneous dc and microwave recordings of single alpha hemolysin/BCD blocking events using glass apertures and coaxial interfaces: shows temporal event correlation while histogram reveals more detail in the microwave recordings vs. conventional patch clamp recordings; this was the primary goal of our Phase I activities.
- Bonded 100 nm Si layers to glass substrates to integrate micromachined probe tips directly with glass apertures
- Constructed new coaxial probe microwave/patch-clamp recording apparatus integrated with magnetic stirrer

Six-Month Milestones

- Integrate tapered Si probe with glass aperture based on new wafer bonding results
- Measure both wild-type (WT) and mutant αHL blocking with βCD using simultaneous microwave and DC recording fixture
- Apply microwave readout technique to other nanoscale apertures in the context of the MOLDICE program

Team Member Organizations

University of Wisconsin, Department of Electrical and Computer Engineering

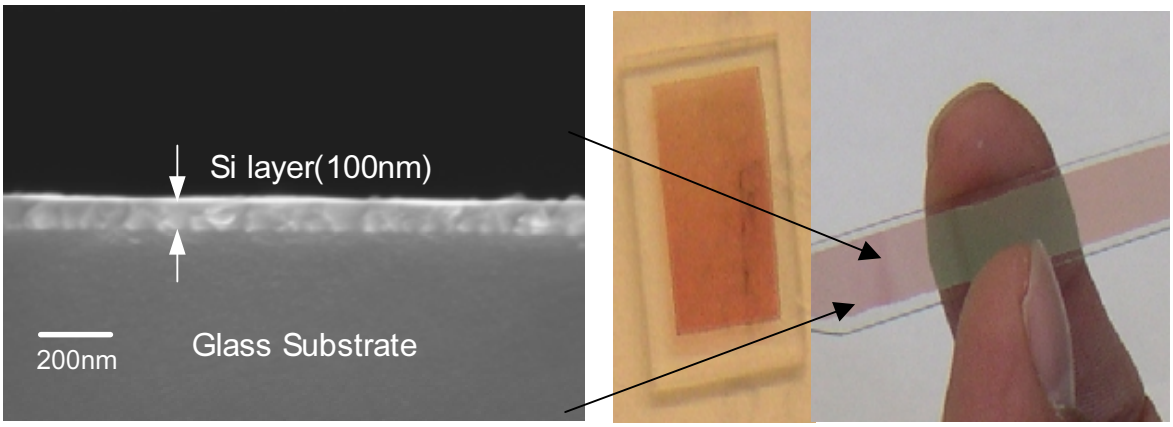


Figure 1: (left) Cross-sectional Scanning Electron Microscope (SEM) image of transferred thin Si layer (100nm) on glass substrate. This structure forms the basis of an integrated Si micromachined tip with glass aperture interface to single membrane proteins. (right) Photograph of the 100 nm transparent Si film on a glass substrate.

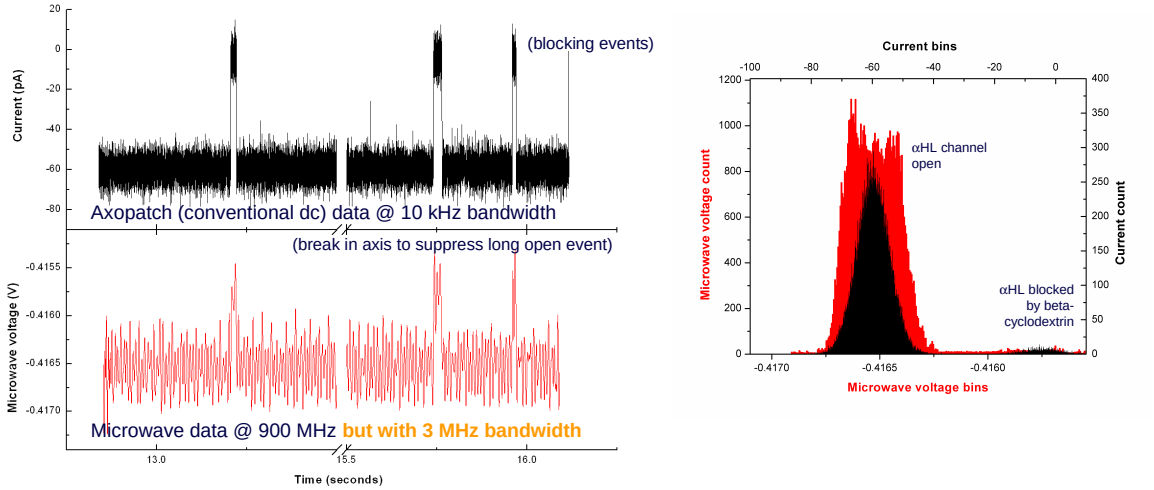


Figure 2: (left): Conventional DC patch-clamp (top) vs. 3 MHz bandwidth 900 MHz microwave recordings (bottom) of βCD blocking WT αHL. (right) Histogram of both recordings (including the long "open" event suppressed in the time-domain presentation). The microwave histogram reveals greater state detail than the DC recording. The microwave transmission is greater when a blocking event occurs, consistent with our model in which a series capacitance increases upon blocking due to the presence of the βCD molecule in the pore. By contrast, of course, the current is reduced upon blocking. Thus the signals are orthogonal.

Publications

Publications

Auburn University

Ion Channel Based Biosensors

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J. C. Sykora, W. C. Neely, & V. Vodyanoy (2004). “Thermodynamic characteristics of mixed monolayersof amphotericin B and cholesterol”. Journal of Colloid and Interface Science, 276, 60-67.

E.M. Josephson, S. Yilma, V. Vodyanoy, and E.E. Morrison (2004)“Structure and Function of Long-LivedOlfactory Organotypic Cultures From Postnatal Mice” Journal of Neuroscience Research 75, 642–653

S. Yilma, J. Cannon, A. Samoylov, T. Lo, N.Liu, C. J. Brinker, W.C. Neely, and V.Vodyanoy, “Large-conductance cholesterol-amphotericin B channels in reconstituted lipid bilayers”. Manuscript submitted in Biochem Biophys Acta.

S.Yilma, N. Liu, A. Samoylov, T. Lo, C. J. Brinker, W. C. Neely, and V.Vodyanoy,”Ampotericin B channels in phospholipid membrane coated nanoporous silicon surfaces: implications for photovoltaic driving of ions across membranes”. Manuscript submitted in Langmuir

T.S. Denney,. K. Wang, S. Yilma, N. Viswaprakash, J. Dennis, V. Vodyanoy, E.E. Morrison, “Olfactory Response Modeling Using System Identification Techniques.” Manuscript submitted in IEEE Transactions on Biomedical Engineering.

Brown University

Direct Nanoscale Conversion of Bio-Molecular Signals into Electronic Information

S.A. Trammell, L. Wang, J. Zullo, R. Shashidhar, N. Lebedev: Oriented Binding of Photosynthetic Reaction Centers on Gold using Ni-NTA Self-Assembled Monolayers.- Biosensors and Bioelectronics, 19:1649–1655, 2004.

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B.J. Taft, A.D. Lazareck, G.D. Withey, A. Yin, J.M. Xu, S.O. Kelley, "Site-Specific Delivery of DNA and Appended Cargo to Carbon Nanotubes." *Accepted to Nano Letter*s, 2004.

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Das,R., Kiley,P.J., Segal,M., Norville,J., Yu,A.A., Wang,L., Trammell,S.A., Reddick,L.E., Kumar,R., Stellacci,F., Lebedev,N., Schnur,J., Bruce,B.D., Zhang,S., Baldo,M.: Integration of photosynthetic protein molecular complexes in solid-state electronic devices. - Nano Letters 4:1079–1083, 2004

B.J. Taft, A.D. Lazareck, G.D. Withey, A. Yin, J.M. Xu, S.O. Kelley, "Site-Specific Delivery of DNA and Appended Cargo to Carbon Nanotubes." *Accepted to Nano Letter*s, 2004.

R. Gasparac, B.J. Taft, M. Lapierre-Devlin, A.D. Lazareck, J.M. Xu, S.O. Kelley, "Ultrasensitive DNA Detection at 2D and 3D Nanoelectrodes." *J. Am. Chem. Soc*., in press, 2004.

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J.M. Xu, (invited), “Nanotube Electronics – Non CMOS Routes”, Proceedings of IEEE, Special Issue on Nanoelectronics and Giga-scale Systems, 91(11), 1819-1829, 2003.

Z. Siwy, E. Heins, C.C. Harrell, P. Kohli, C.R. Martin, Conical-Nanotube Ion-Current Rectifiers – The Role of Surface Charge, *J. Am. Chem. Soc.* (2004) published on-line.

C.C. Harrell, P. Kohli, Z. Siwy, C.R. Martin, DNA-Nanotube Artificial Voltage-Gated Ion Channel, submitted to *J. Am. Chem. Soc.*

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Yeh, J.I., Hou, L., and Zimmerman, A. (2004). “Structure of a 33-Amino Acid Peptide Nanowire”, in preparatio*n.*

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Paulo, J., Zhang, S. and Yeh, J.I. “Functional Characterization of a Membrane-Associated Protein Solubilized with a Surfactant-Like Peptide”, in preparation.

Conference papers

Presentation at the 205 th Meeting of The Electrochemical Society, May 9-13, 2004, San Antonio, TX: C.C. Harrell, P. Kohli, Z. Siwy, E. Heins, and C.R. Martin, Mimicking Voltage-Gated Ion Channel Analogues Using Robust Abiotic Membranes.

Presentation at the PITTCON March12, 2004, Chicago: C.C. Harrell, P. Kohli, and C. R. Martin, Ion channel mimetic synthetic single nanotube membranes.

J.M. Xu, (Invited), “Interfacing Biomolecules with Nanoelectronics”, Gordon Conference on Computational Chemistry, July 4-9, 2004, New Hampshire.

J.M. Xu, (Invited), “Bio-nanoelectronics”, Seoul National University, Seoul, Korea, August 26, 2004

Control and System Integration of Micro and Nano-Scale Systems, Report from the National Science Foundation workshop, Washington, DC, March 29–30, 2004 p.87

Strategic Research to Enable NASA’s Exploration Missions Conference, Cleveland, Ohio, June 22-23, 2004 p.103

Patents

C. Martin, et. al.“Chemical and Biosensing with Nanopore and Nanotube Membranes”, in preparation.

S. Kelley et. al., “Electrocatalytic DNA Detection”, PCT application filed 5/04.

N. Kouklin and J.M. Xu, “Controlled Assembly of Molecular-Scale Carbon Nanotube Probes”, filed 2/04.

J. Yeh, et. al., Biomolecular Conduits of Electronic Signals (in preparation)

Impact on teaching (new course, or new content in an existing course)

Lebedev’s group includes 5 summer students (3 in NRL and 2 in UVa)

Kelley runs a literature workshop over the last six months for graduate students focused on advances in the uses of nanomaterials for biosensing.

Xu’s group has 2 undergraduate students and 12 graduate students.

Awards

Sloan Research Fellowship to Kelley; Kelley named as Technology Review’s “Top 100 innovator”

Ionata Award for the most creative thesis to Karl Hanson (Xu’s group)

Rush Medical Center

Ionic Channels as Natural Nanodevices

Reviews

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Gillespie, D., Nonner, W. and RS Eisenberg. (2003) Crowded Charge in Biological Ion Channels Nanotech 3: 435

Nadler, B., Schuss, Z., Singer, A., Eisenberg, B. (2003) Diffusion through protein channels: from molecular description to continuum equations. Nanotech 3: 439

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Goryll, M., Wilk, S., Laws, G. M., Thornton, T. J., Goodnick, S. M., Saraniti, M., Tang, J. M. and R. S.Eisenberg (2003) Silicon-based ion channel sensor Superlattices & Microstructures 34(3-6), 451-457.

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University of Florida

Array-Based Nanopore Stochastice Sensors for Mutilplexed Bioassays

Gasparac, R.; Kohli, P.; Paulino, M.O.M.; Trofin, L.; Martin, C.R. “Template Synthesis of Nano Test Tubes,” *Nano Letter*s, 2004, 4, 513-516.

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University of Wisconsin

Single-Receptor Interfaces for Real-time Kinetics

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‘Single-channel recordings with microwaves’, S. Ramachandran, I.-S. Son, K. Taylor, H.-S. Kim, R.H. Blick, and D.W. van der Weide, in preparation, October (2004).